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CEREBRAL MICROBLEEDS AND COGNITIVE IMPAIRMENT

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Cerebral Microbleeds and Cognitive Impairment

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To everyone wishing for a dedicated PhD thesis

"*When swimming with sharks, don't bleed when bitten*"

— Peter Aspelin

ABSTRACT

Background: With increasingly ageing populations comes an increased prevalence of cognitive impairment and dementia. The pathophysiology behind dementia is still unknown, and there is no cure. Microscopic bleeds in the brain parenchyma, so called cerebral microbleeds, are common in ageing populations, as well as in dementia and stroke, and are primarily a marker of small vessel disease. Due to their high prevalence in memory clinic populations, microbleeds have been hypothesized to be of importance in the cognitive impairment disease process.

Purpose: To cross-sectionally study the detection and clinical implications of cerebral microbleeds in cognitive impairment.

Study I showed that microbleeds are common in cognitive impairment (22% prevalence), and especially in vascular dementia (59% prevalence). Microbleeds are associated with hypertension, male gender, high age, and increase with increasing risk factors. Topography of microbleeds is predominantly lobar and occipital in Alzheimer disease. The microbleed topography varies depending on underlying diagnosis and risk factors.

Study II showed that susceptibility weighted imaging increases the prevalence and number of microbleeds detected on 3.0T magnetic resonance imaging. Inter-rater agreement of microbleeds is excellent on T2* and susceptibility weighted imaging, across raters of different experience. Only minor differences in clinical associations were noted across different sequences.

Study III showed that amyloid β42 levels were lower in the cerebrospinal fluid with a high number of microbleeds. This was true in the whole cohort (n=1039), Alzheimer disease and mild cognitive impairment. In the whole cohort cerebrospinal fluid/serum albumin ratios were higher with increasing number of microbleeds. In multivariate regression analysis low amyloid levels in the cerebrospinal fluid with increasing number of microbleeds held true. White matter hyperintensities were likewise associated with low amyloid β42 levels, whereas lacunes were associated with higher amyloid levels in the cerebrospinal fluid.

Study IV showed that lobar microbleeds are associated with lower amyloid β42 levels in the cerebrospinal fluid, in the whole cohort and Alzheimer disease. Deep and infratentorial microbleeds showed tendencies to be associated with higher amyloid and lower tau levels in the cerebrospinal fluid. Multivariable logistic regression analysis showed that white matter hyperintensities and lacunes were associated with lobar and deep microbleeds.

Conclusions: Cerebral microbleeds are best detected with susceptibility weighted MRI and are common in a memory clinic. Microbleeds show varying associations based on topography. Especially lobar microbleeds are associated with low cerebrospinal fluid amyloid, and specifically in Alzheimer disease, suggesting that primarily lobar microbleeds may be of importance in cognitive impairment.

SAMMANFATTNING

Bakgrund: Kognitiv svikt samt demens ökar i takt med att befolkningsåldern globalt ökar. Patofysiologin bakom demens är fortfarande okänd och det finns inget bot. Mikroskopiska blödningar i hjärnan, så kallade cerebrala mikroblödningar, är en markör för småkärlssjuka, och är vanliga i åldrande populationer, samt hos patienter med demens och stroke. Till följd av deras höga prevalens i grupper med stroke samt demens har mikroblödningar ansetts vara av vikt i sjukdomsprocessen hos patienter med kognitiv svikt.

Syfte: Att i tvärsnittsstudier utforska cerebrala mikroblödningar inom kognitiv svikt, deras implikationer, associationer och detektionsmetoder.

Studie I visar att mikroblödningar är vanligt förekommande inom kognitiv svikt (22% prevalens), och är som mest vanligt inom vaskulär demens (59% prevalens). Mikroblödningar är associerade med hypertension, manligt kön och hög ålder, och ökar med ökande antal riskfaktorer. Mikroblödningar är främst lokaliserade i hjärnloberna, och främst occipitalt inom Alzheimers sjukdom. Mikroblödningslokalisation varierar beroende på underliggande orsak och association med riskfaktorer.

Studie II visar att susceptibilitets-viktade sekvenser leder till ökad prevalens och ökat antal detekterade mikroblödningar på 3.0T magnetresonanstomografi. Överensstämmelse mellan raters av mikroblödningar är utmärkt på T2* samt susceptibilitets-viktade sekvenser, även hos raters med olika erfarenhet. Enbart små skillnader i kliniska associationer till mikroblödningar noterades för de olika magnetkamera-sekvenserna.

Studie III visar att amyloid β42-nivåer minskar i cerebrospinalvätska med ökat antal mikroblödningar, för hela kohorten, Alzheimers sjukdom och lindrig kognitiv svikt. Ration för albumin i cerebrospinalvätska/serum var högre med ökande antal mikroblödningar. Låga amyloid β42-nivåer i cerebrospinalvätska var associerade med ökande antal mikroblödningar samt vitsubstansförändringar. Lakuner var associerade med höga amyloid β42-nivåer i cerebrospinalvätska.

Study IV visar att mikroblödningar i hjärnloberna är associerade med lägre amyloid β42-nivåer i cerebrospinalvätska, i hela kohorten samt Alzheimers sjukdom. Djupa och infratentoriella mikroblödningar visar tendenser till att vara associerade med högre amyloid β42- och lägre tau-nivåer i cerebrospinalvätska. Multivariabla logistiska regressionsanalyser visar att vitsubstansförändringar och lakuner är associerade med mikroblödningar i hjärnlober samt djupa områden i hjärnan.

Slutsats: Cerebrala mikroblödningar är bäst detekterade med susceptibilitets-viktade sekvenser och är vanliga inom en minnesklinik. Mikroblödningar och olika associationer varierar beroende på blödningens lokalisation. Särskilt mikroblödningar i hjärnloberna visar associationer till låga värden av amyloid i cerebrospinalvätska, och specifikt inom Alzheimers sjukdom. Sannolikt är primärt mikroblödningar i hjärnloberna av vikt hos patienter med kognitiv svikt.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following four papers, which will be referred to in the text by their roman numerals.

I. **Cerebral microbleeds: different prevalence, topography, and risk factors depending on dementia diagnosis—the Karolinska Imaging Dementia Study.**
Shams S, Martola J, Granberg T, Li X, Shams M, Fereshtehnejad SM, Cavallin L, Aspelin P, Kristoffersen-Wiberg M, Wahlund LO. *AJNR Am. J. Neuroradiol. 2015;36:661–666.*

II. **SWI or T2*: which MRI sequence to use in the detection of cerebral microbleeds? The Karolinska Imaging Dementia Study.**
Shams S, Martola J, Cavallin L, Granberg T, Shams M, Aspelin P, Wahlund LO, Kristoffersen-Wiberg M.
AJNR Am. J. Neuroradiol. 2015;36:1089–1095.

III. **Cerebrospinal fluid profiles with increasing number of cerebral microbleeds in a continuum of cognitive impairment.**
Shams S, Granberg T, Martola J, Li X, Shams M, Fereshtehnejad S-M, Cavallin L, Aspelin P, Kristoffersen-Wiberg M, Wahlund L-O.
J. Cereb. Blood Flow Metab. 2015; 36(3):621-8.

IV. **Cerebral microbleeds topography and cerebrospinal fluid biomarkers in cognitive impairment.**
Shams S, Granberg T, Martola J, Charidimou A, Li X, Shams M, Fereshtehnejad S-M, Cavallin L, Aspelin P, Kristoffersen-Wiberg M, Wahlund L-O.
J. Cereb. Blood Flow Metab. 2016; E-pub ahead of print.

CONTENTS

LIST OF ABBREVIATIONS

Aβ	Amyloid β
AD	Alzheimer Disease
ARD	Alcohol related dementia
ANOVA	Analysis of variance
BBB	Blood brain barrier
CAA	Cerebral amyloid angiopathy
CMBs	Cerebral microbleeds
CSF	Cerebrospinal fluid
CT	Computed tomography
FLAIR	Fluid attenuated inversion recovery
FDG	Fludeoxyglucose
FTD	Frontotemporal lobe dementia
IQR	Interquartile range
KIDS	Karolinska Imaging Dementia Study
LBD	Lewy body dementia
MARS	Microbleed anatomical rating scale
MCI	Mild cognitive impairment
MMSE	Mini mental state examination
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangles
OR	Odds ratio
PACS	Picture archiving communicating system
PDD	Parkinson disease dementia
PET	Positron emission tomography
PiB	Pittsburgh compound B
SCI	Subjective cognitive impairment
SD	Standard deviation
SDMT	Symbol digit modalities test
SVD	Small vessel disease
TE	Time to echo

VaD	Vascular dementia
WMH	White matter hyperintensities

1 INTRODUCTION

1.1 COGNITIVE IMPAIRMENT

Cognitive impairment is a loss in cognitive function, whether subjective or objectively observed. Dementia, prestages of dementia and subjective cognitive impairment (SCI) may all be termed cognitive impairment. With increased ageing of populations, the prevalence of cognitive impairment, and especially Alzheimer disease (AD), is expected to rise[1,2]. In 2010 35.6 million people lived with dementia worldwide; this number is expected to approximately double every 20 years[3]. The total estimated worldwide cost of dementia is US$ 818 billion[4]. However, disease pathology still remains elusive, and there is currently no treatment. Figure 1 shows the global impact of dementia.

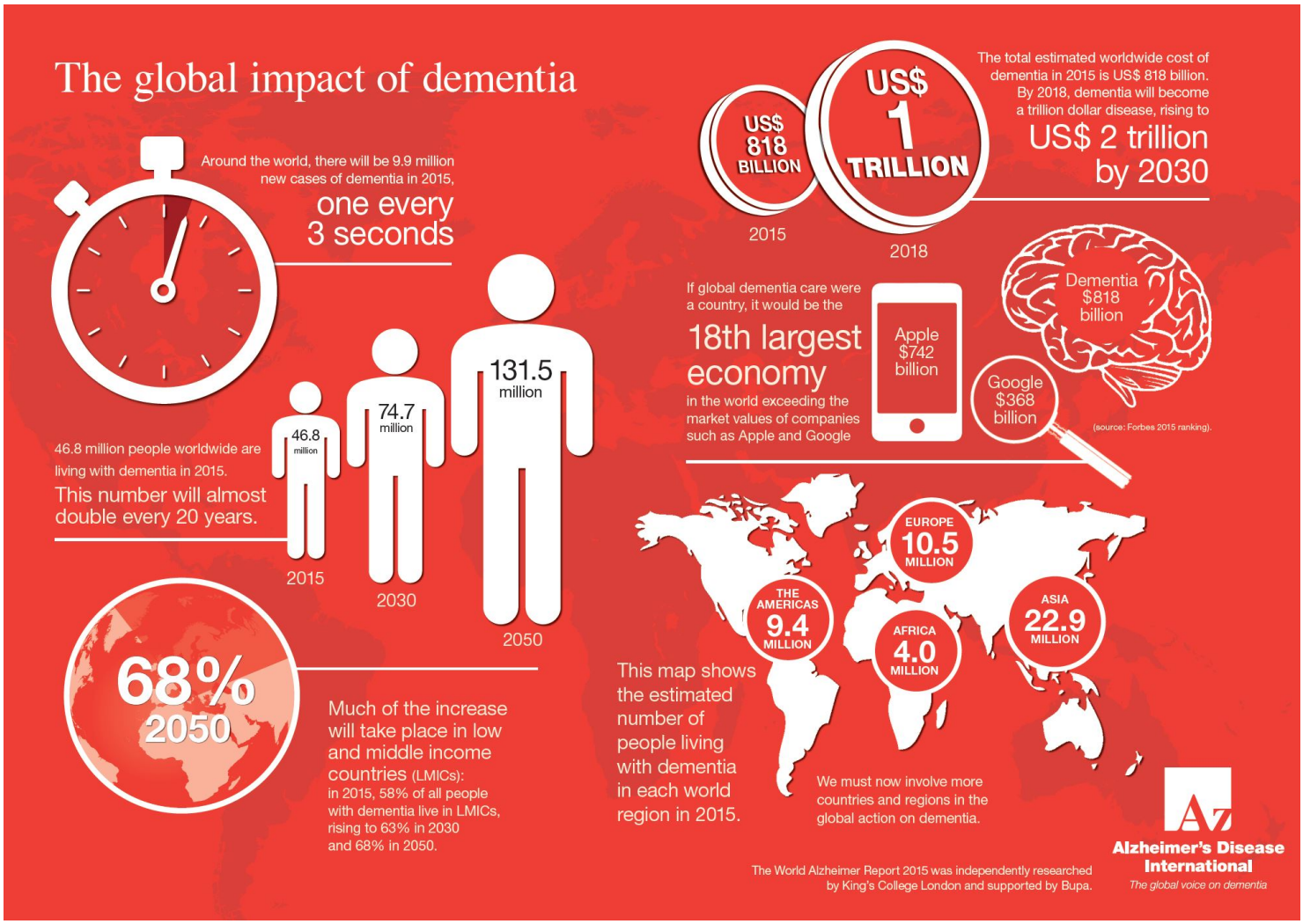

Figure 1. The global impact of dementia. Reprinted by permission from Alzheimer's disease international, World Alzheimer Report 2015.

1.1.1 Diagnoses in a memory clinic

AD is the most common form of dementia and accounts for 60-80% of dementias[5,6]. The hallmarks of AD are amyloid plaques, consisting of a core of amyloid β (Aβ) 42, and neurofibrillary tangles (NFT) composed of paired helical filaments and hyperphosphorylated tau[5]. The pathophysiology of AD remains elusive. Despite several hypotheses proposed, such as the amyloid cascade hypothesis, cholinergic hypothesis, inflammatory hypothesis, and the vascular hypothesis, no hypothesis has exhaustively and rationally been able to delineate AD pathophysiology[7–10]. Diagnosis of AD is based on the clinical presentation of dementia and cognitive decline with emphasis on amnesia[11]. Increased certainty may be added through the use of cerebrospinal fluid (CSF) analysis of biomarkers Aβ 42, total tau (T-tau) and phosphorylated tau (P-tau)[12] as well as imaging. Recently, the research based AD criteria were revised to increase simplicity and availability of diagnosis, as well as incorporating biomarkers such as neuropsychological testing and imaging to improve diagnostic certainty[13]. Figure 2 shows positron emission tomography (PET) amyloid imaging with the Pittsburgh compound B (PiB) and fludeoxyglucose (FDG)[14], which may aid diagnosis of AD, similar to CSF biomarkers. Treatment to date consists of acetylcholinesterase inhibitors and N-Methyl-D-aspartate (NMDA) receptor antagonists[10]. Novel anti-amyloid, as well as anti-tau therapeutics are now under trial, and may prove to be important assets in the treatment of AD[5,15].

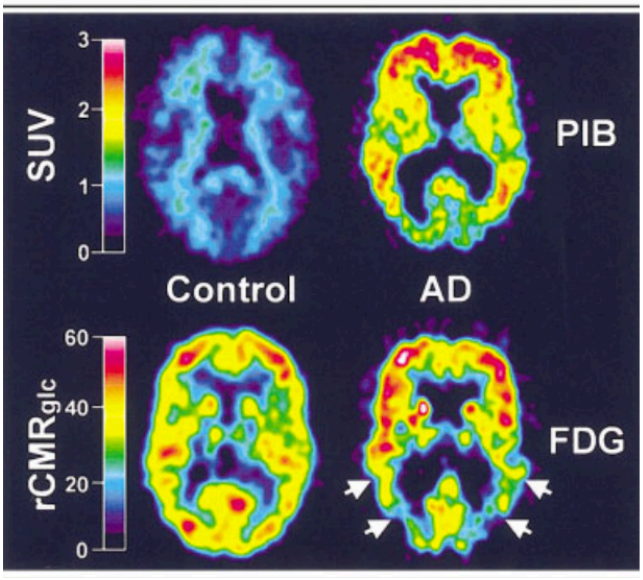

Figure 2. Amyloid deposition as seen by PiB and glucose uptake, as represented by FDG in Alzheimer disease and healthy controls. Reprinted by permission from John Wiley and Sons: Annals of Neurology[14], copyright 2004.

Mild cognitive impairment (MCI) includes the symptomatic prestage of AD. AD pathology is thought to begin years, and possibly decades, before the presentation of clinical symptoms[16,17]. Diagnostic criteria of MCI include cognitive deterioration but without significant impairment limiting life[16,18]. Far from all patients with MCI convert to AD, and it is still unclear what causes progression to dementia. Subjective cognitive impairment is as the

name suggests a cognitive impairment that cannot be objectively verified, but is solely subjective[19]. Subjective cognitive impairment (SCI) has been suggested to be the very preclinical stage of AD, although the group is undeniably diverse, and not everyone progresses to AD[16,19]. Figure 3 and 4 show the hypothesized progression from the preclinical stage to dementia.

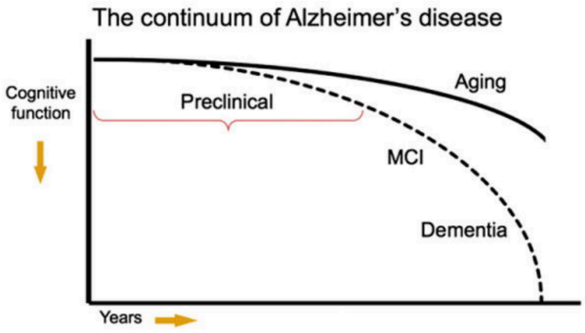

Figure 3. The progression to dementia. Reprinted by permission from Elsevier: Alzheimer's & Dementia[16], copyright 2004.

Lewy body dementia (LBD) is thought to be the second most common dementia, constituting around 10-15% of all dementias[20], although it is debatable whether the second position is shared with vascular dementia or not[21]. The hallmarks of LBD consist of α-synuclein Lewy bodies, and clinical features include visual hallucinations and parkinsonism[20,22]. Parkinson disease dementia (PDD) is distinguished from LBD by the onset of dementia; if dementia presents within 12 months of parkinsonism the diagnosis is considered to be LBD, whereas more than 12 months of parkinsonism before dementia qualifies as PDD[20,22].

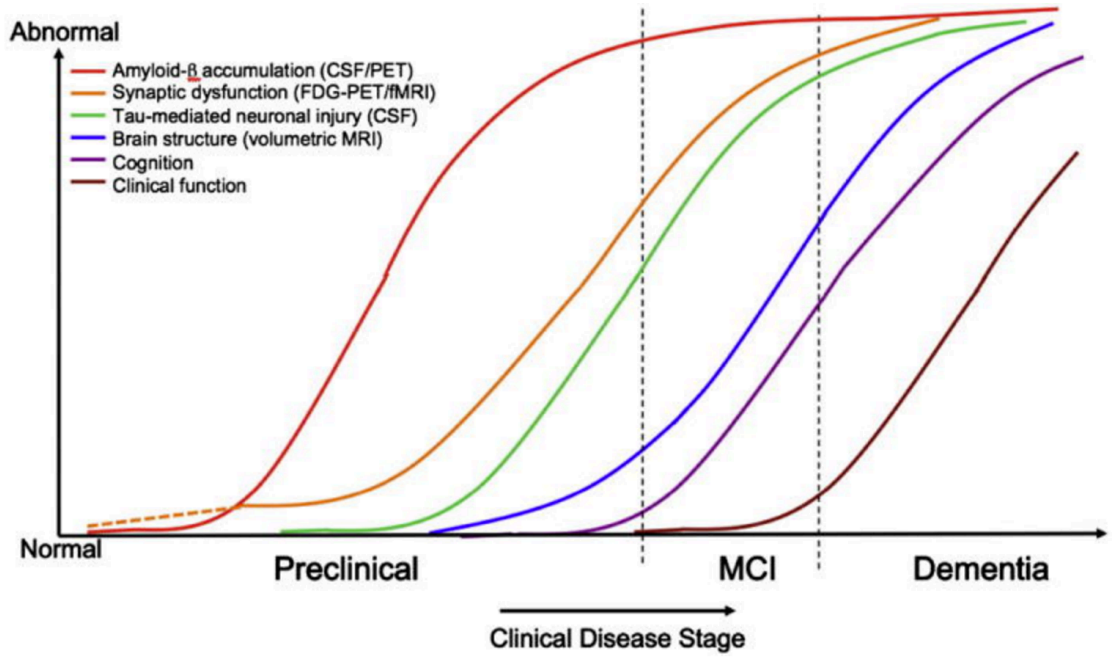

Figure 4. Biomarkers in time during the progression to dementia. Reprinted by permission from Elsevier: Alzheimer's & Dementia[16], copyright 2004.

Vascular dementia (VaD) is in turn also considered to be one of the most common dementias second to AD[23]. It defines all dementias resulting from a vascular pathology[23]. However, depending on the definition, it may be argued that the term vascular dementia has, with the dawn of increased small vessel disease (SVD) research, become obsolete. There is a considerable overlap between VaD and AD,[24] and most, if not all dementias can be argued to

have a vascular component. Cerebral amyloid angiopathy (CAA), amyloid deposition in the media and intima of small vessel walls is thought to be present in healthy ageing and almost all patients with AD[25–27]. Cognitive decline due to CAA has been seen[26,28–30]. The classification of VaD includes: strategic-infarct dementia, cortical vascular dementia, subcortical ischemic dementia, hypoperfusion dementia, hemorrhagic dementia and dementias resulting from arteriopathies[23]. Primary prevention, by early on attacking cardiovascular as well as cerebrovascular risk factors, is what is practiced and thought to reduce the incidence of VaD[23,31].

Frontotemporal lobe dementia (FTD) is characterized by selective degeneration and atrophy of the frontal and temporal lobes[32]. Prevalence is considered less than the above-mentioned dementias; studies have shown a prevalence range of 4-15/100 000 in populations younger than 65 years[32,33]. Disease presentation often occurs in the third to ninth decade of life, although around the sixth decade is more common[32]. Three clinical variants of FTD have been described, behavioral variant FTD, semantic dementia, and progressive nonfluent aphasia[33].

Alcohol related dementia (ARD) and Wernicke-Korsakoff syndrome are both a result of excessive alcohol consumption. ARD is still debated as whether the effects are due to ethanol toxicity in itself or the related lack of nutrition, vitamin deficiencies (e.g. thiamine), the life style with increased risk of head trauma, and the higher number of vascular risk factors[34]. Thiamine deficiency is the cause of Wernicke encephalopathy, characterized by the classical triad ophthalmoplegia, ataxia and dementia[34]. Korsakoff syndrome, caused by thiamine deficiency, in turn denotes an acute onset of cognitive decline, and often occurs together with Wernicke encephalopathy, hence the name Wernicke-Korsakoff syndrome[34]. Treatment includes cutting down on, or giving up, alcohol, as well as high doses of thiamine[34].

Other diseases, and secondary causes of cognitive impairment also exist in a memory clinic. Creutzfeldt-Jakob disease, and cognitive impairment associated with other disease panoramas such as multiple sclerosis, aids and amyotrophic lateral sclerosis, may also surface in a memory clinic. Secondary causes of cognitive impairment include, amongst others, subdural hematoma and slow growing tumors. Clinical dementia with an unknown cause is termed unspecified dementia.

1.1.2 Cerebrospinal fluid measurements

CSF analysis, and thus a lumbar puncture, is done routinely in memory clinic investigations in Sweden. It yields important differential diagnostic data in the reasoning of diagnosis. Since the CSF is in constant direct contact with the brain it also reflects the biochemical state of the brain[12]. Biomarkers usually analyzed include Aβ 42, T-tau, P-tau and CSF/serum albumin ratios. A low Aβ 42 level in the CSF is thought to reflect increased amyloid deposition in the brain, and is what is expected in AD[35,36]. High T-tau and P-tau levels are commonly seen in AD, although unspecific, they reflect neurodegeneration and NFTs respectively[35]. CSF/serum

albumin ratios reflect the integrity of the blood brain barrier (BBB), increases in the ratio reflecting increased permeability[37].

1.1.3 Neuropsychological testing

Neuropsychological testing is routinely done in memory clinic investigations. Episodic memory deficits are usually the first line of cognitive impairment in AD[11]. Semantic memory impairment, as well as concentration and visuospatial difficulties may also occur in AD[11]. The mini mental state examination (MMSE) is an easy and efficient screening tool for cognitive impairment. Due to the ease in which it can be clinically used it is one of the most common tests of cognitive screening, and the maximum score is 30[38]. MMSE tests language, memory, attention and figure copying amongst others[38,39].

1.1.4 Imaging

Imaging has gained importance in the diagnostic reasoning, and is increasingly a cornerstone in memory clinic investigation. Computed tomography (CT) of the brain is quick and efficient, and still probably the most used modality as part of memory clinic investigations worldwide. Magnetic resonance imaging (MRI) shows a great level of detail, and is increasingly replacing CT as an examination. MRI is ideal for maximal differential diagnostic reasoning, and makes imaging of SVD markers possible.

With imaging it is of importance to first rule out secondary causes of dementia such as an expansive mass or hydrocephalus. In detailed assessment of cognitive impairment, atrophy may be evaluated with the use of different rating scales[40]. Rating scales for both CT and MRI include the global cortical atrophy scale,[41] the Koedam scale assessing for parietal atrophy, the medial temporal atrophy scale, and the Fazekas[42] or the age related white matter changes scale[43]. PET imaging is of use in the differential diagnostic reasoning, and FDG PET is the most commonly used tracer. Additional assessment can be done on MRI, where markers of SVD such as cerebral microbleeds (CMBs), lacunes, enlarged perivascular spaces, cortical superficial siderosis and white matter hyperintensities (in a higher level of detail)[30], may be analyzed. Figure 5 depicts the distribution of CAA and related SVD markers.

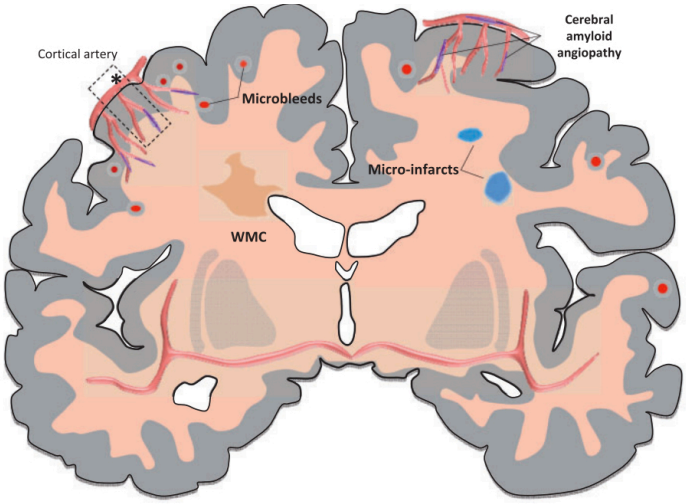

Figure 5. CAA related markers in the brain. Reprinted by permission from Oxford University Press: Brain[44], copyright 2011, and Dr Andreas Charidimou.

1.2 CEREBRAL MICROBLEEDS

1.2.1 Etiology

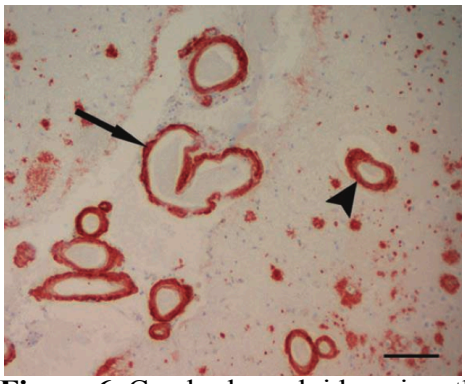

Figure 6. Cerebral amyloid angiopathy seen in vessels stained with congo red. Reprinted by permission from John Wiley and Sons: Neuropathology and applied neurobiology[45], copyright 2012.

Microscopic bleeds in the brain parenchyma, CMBs, most frequently arise from SVD. SVD is the disease of microscopic vessels in the brain[30]. The two most common etiologies of SVD are: 1. CAA, which is amyloid deposition in the media and intima of vessel walls, leading to vessel fragility, disruption of the vessel walls, possible microaneurysms, blood extravasation and sometimes also luminal occlusion[30]. Figure 6 shows a histopathological image of CAA. 2. Hypertensive arteriopathy is hypertensive related damage, primarily affecting the deep perforating vessels[30,45]. Pathophysiology includes arteriolosclerosis, fibrohyaline deposits narrowing the lumen, thickening of the vessel wall, atherosclerosis and microaneursyms, amongst others[30,45]. CAA and hypertensive arteriopathy are thought to have different locations, with CAA mainly affecting the brain lobes and hypertensive arteriopathy the deep regions of the brain[30,45]. Figure 7 depicts the pathophysiology of CAA. Since SVD affects small vessels in the brain, which cannot be imaged per se, imaging markers of SVD are used as signatures of the disease[30], seen in Figure 8. CMBs are one imaging marker of SVD. Their topography follows that of SVD, with lobar CMBs representing CAA, and deep CMBs, hypertensive arteriopathy.

1.2.2 Detection

CMBs can only be detected in vivo by MRI, and are usually only seen with hemosiderin sensitive sequences such as the T2* and susceptibility weighted imaging (SWI) sequence[29,45]. Since CMBs are supraparamagnetic, they introduce inhomogeneities in the magnetic field, causing rapid decay of the MRI signal, termed the susceptibility effect[29,46]. This leads to CMBs having a hypointense appearance on MRI[29]. MRI parameters affect the detection of CMBs; for instance an increased time to echo (TE) leads to increased time for dephasing and an enlarged susceptibility effect, i.e. appearance of CMBs[29,47]. Other ways of increasing CMB detection include the use of SWI, higher field strength and thinner slice thickness[29,48–50]. The microbleed anatomical rating scale (MARS) is a standardized rating scale for CMBs[51]. Mimics for CMBs, that are detailed in the MARS, and need to be avoided in CMB rating include calcifications, cross-sectioned vessels, partial volume artifacts and cavernomas, amongst others[29,51]. Histopathological studies have shown a good correspondence between CMBs on MRI and histopathology[52].

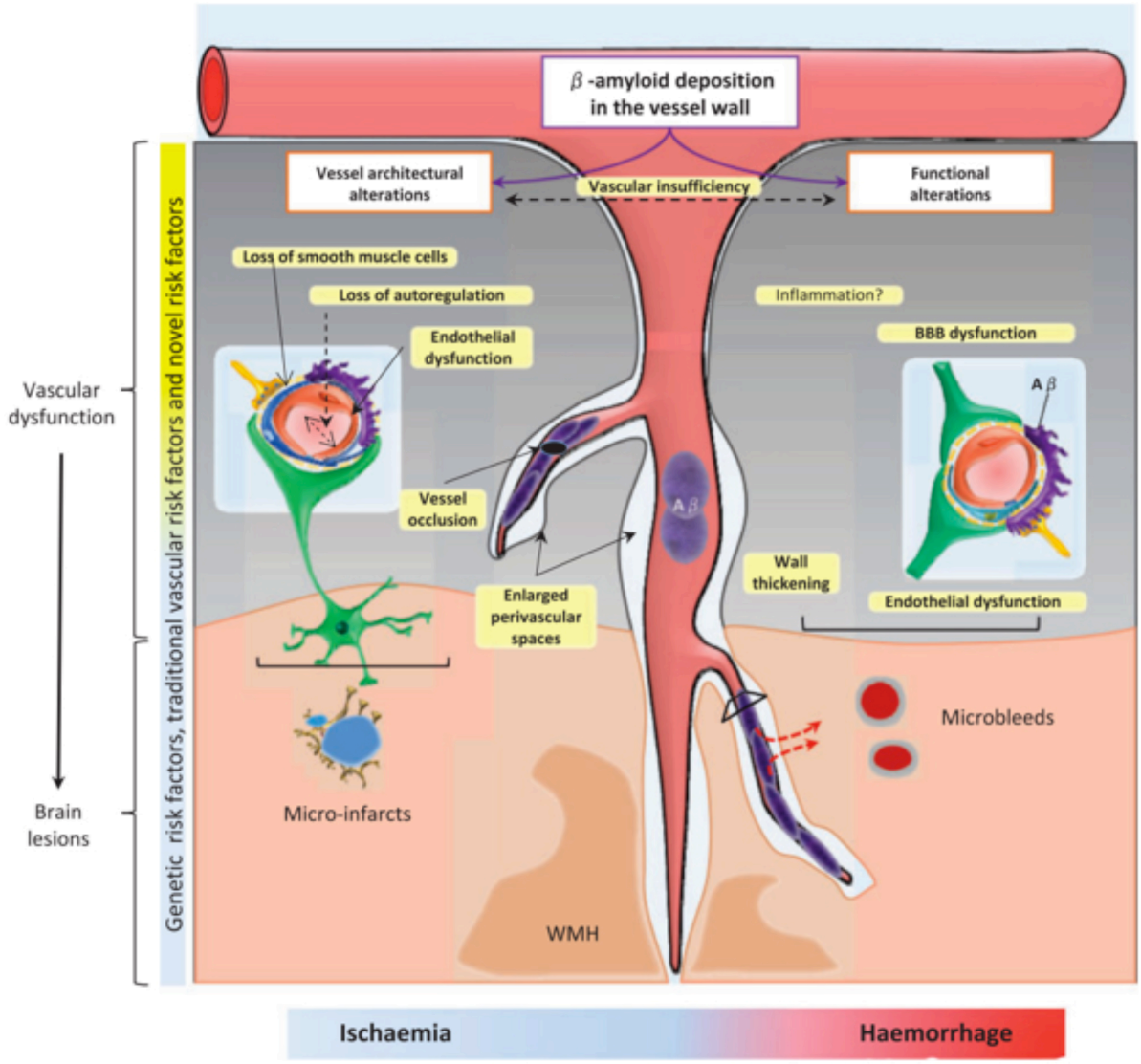

Figure 7. The pathophysiology and markers of CAA. Reprinted by permission from Oxford University Press: Brain[44], copyright 2011.

1.2.3 Implications

CMBs have been detected since the 1990s, with the use of hemosiderin sensitive sequences[29,53,54]. Initially hemosiderin sequences were only included for research purposes, and it took time before the sequences were incorporated in clinical MRI protocols. At the radiology department at the Karolinska university hospital it was first in and around 2006 that hemosiderin sensitive sequences were introduced in routine MRI protocols. It was quickly noted that especially two populations had CMBs more frequently than others: patients with cognitive impairment and stroke. At that time CMBs were still a dilemma, and their clinical implications remained unknown. The worldwide increased detection, especially in these two populations, prompted a surge of research on the topic, such as this PhD thesis.

The higher frequency of CMBs in memory clinic populations, around 18-32%[55–59], when compared to healthy ageing populations, with a prevalence usually in the range of 6-11%[60–62], suggests an involvement of CMBs in cognitive impairment[63]. CMBs were hypothesized to bridge the vascular theory in AD and the amyloid hypothesis[63]. The hypothesis is that abnormal amyloid precursor protein cleavage may lead to abnormal accumulation of Aβ in vessel walls and thus vessel fragility; at the same time atherosclerosis/arteriolosclerosis would contribute to decreased vessel wall integrity. Both these processes would eventually lead to CMBs. The blood extravasation and vessel wall fragility would open up for influx of plasma components, that would trigger neurodegeneration, and eventually AD[63]. Further hypotheses suggest that amyloid plaques stem from the vasculature and align with CMBs[64,65].

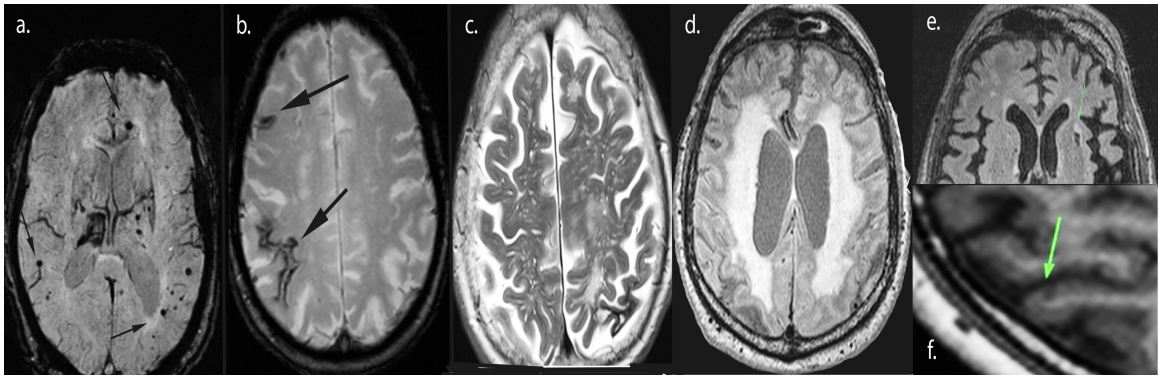

Figure 8. MRI markers of SVD, which are in order: a) Cerebral microbleeds b) Cortical superficial siderosis c) Enlarged perivascular spaces. d) White matter hyperintensities. e) Lacune. f) Cortical microinfarct.

2 AIMS OF THIS THESIS

With increased detection of CMBs, due to incorporation of hemosiderin sensitive MRI sequences in routine clinical protocols, questions were raised. The clinical implications of CMBs were unknown, and the fact that they were more frequent in memory clinic populations raised hypotheses that they may interact with the neurodegenerative process in cognitive impairment. The purpose of this thesis was to investigate CMBs in a memory clinic population, its prevalence, detection method, implications and associations.

The specific objectives of each study were:

Study I To study the prevalence, topography and associations as well as clinical implications of CMBs in a memory clinic.

Study II To determine which MRI sequence should be used in the rating of CMBs, if the SWI and T2* are comparable with regards to inter-rater agreement and clinical associations with CMBs, across sequencs.

Study III To determine how CSF biomarkers (Aβ 42, T-tau, P-tau and CSF/serum albumin ratios) are associated with CMBs, and to see if: 1. Associations between biomarkers and CMBs increase in the continuum of cognitive impairment from SCI to AD. 2. Biomarkers reach pathological levels with increased numbers of CMBs. 3. The joint presence of amyloid pathology and hypertensive arteriopathy would pronounce the relation with CSF biomarkers.

Study IV To study: 1. The association between CSF biomarkers and CMB topography. 2. The prediction by other MRI markers of SVD and CSF biomarkers, in the likelihood of having lobar versus deep/infratentorial CMBs.

3 MATERIALS AND METHODS

3.1 ETHICAL CONSIDERATIONS

For all studies: Informed consent was obtained from each patient, according to the declaration of Helsinki. Ethical approval was obtained from the regional ethical board, Stockholm, Sweden (registration numbers: 2012/2038-32, 2013/363-32).

3.2 PATIENTS

This thesis is the first thesis in the cross-sectional Karolinska Imaging Dementia Study (KIDS). The KIDS aims to investigate SVD and other imaging markers in a memory clinic population. Inclusion criteria for the cohort used in Studies I, III and IV were patients undergoing memory clinic investigation with an accompanying MRI scan, and an MRI protocol including hemosiderin sensitive sequences (SWI/ T2*). Exclusion criteria were insufficient scan quality or prior history of brain trauma. A total cohort of 1504 patients were enrolled, encompassing 10 diagnostic groups. In study III and IV inclusion criteria was restricted to the above, and additionally CSF biomarker analysis leading to a cohort of 1039 patients. In study II, the inclusion criteria were restricted to patients with both the SWI and T2* on 3.0T MRI imaging, yielding a cohort of 246 patients. Diagnoses were set according to the ICD-10[66] in multidisciplinary rounds, with consideration of all data, such as imaging, lab tests, neuropsychological testing, and a routine clinical work up. All diagnoses and the ICD-codes associated are seen below in Table 1. All patients' clinical notes were analyzed and data extracted. For instance, to classify as hypertensive the patient either had to have the diagnosis in their medical record, or the appropriate medication.

Table 1. Diagnoses and accompanying ICD-codes

Diagnosis (n=1504)	ICD-codes
Subjective Cognitive Impairment (n=385)	Z03.2A, Z03.3 and R41.8A
Alcohol Related Dementia (n=20)	F10.6, F10.7a
Alzheimer's Disease (n=423)	F00.0 (early onset, n=176), F00.1 (late onset, n=146), F00.2 (atypical disease with vascular components, n=96), F00.9 (unspecified Alzheimer's disease, n=5)
Asymptomatic Hereditary Dementia (n=45)	Z31.5
Frontotemporal Lobe Dementia (n=30)	F0.70, F02.0
Mild Cognitive Impairment (n=418)	F06.7
Parkinson's Dementia (n=21)	F02.3, G31.8a
Unspecified Dementia (n=55)	F03.9
Vascular Dementia (n=54)	F01.1, F01.2, F01.3, F01.9 and CADASIL (4 patients) based on I63.8
Other Disorders (n=53)	Depression, hallucination, delirium, other reactions to severe stress, psychosis, bipolar disease, amnesia, systemic lupus erythematosus encephalopathy, dysphasia, degenerative diseases in the basal ganglia, hydrocephalus, narcolepsia, Creutzfeldt Jacob disease, supratentorial epidermoid tumour, cerebral infarctions, anemia, hereditary ataxia, multiple system degeneration and progressive supranuclear palsy.

3.3 MAGNETIC RESONANCE IMAGING

All patients had their MRI done at the radiology department at the Karolinska university hospital, Stockholm, Sweden. Patients were scanned in three different scanners (Siemens Medical Systems, Erlangen, Germany) noted in Table 2. All patients had a T1, T2, FLAIR, diffusion weighted sequences as well as the SWI and/or T2* sequences.

Table 2. MRI parameters for the T2*/SWI.

Siemens Magnetom		Symphony	Avanto	Trio
Field strength (T)		1.5	1.5	3.0
T2*	Time to echo	25	26	20
	Time to repeat	792	800	620
	Flip angle	20°	20°	20°
	Slice thickness	5.0	5.0	4.0
SWI	Time to echo	-	40	20
	Time to repeat	-	49	28
	Flip angle	-	15°	15°
	Slice thickness	-	4.0	1.6
Patients (n)	-	453	681	370

3.4 RADIOLOGICAL ASSESSMENT

All images were assessed for CMBs, WMH and lacunes according to standardized criteria[51,67]. CMBs were assessed according to the MARS[51] as rounded hypointensities in the brain parenchyma on axial SWI/T2* sequences. Care was taken to avoid rating mimics such as calcifications, cross-sectioned vessels, partial volume artifacts and cavernomas. For instance hypointensities in the globus pallidus were not rated and were regarded as physiological iron deposition/calcifications. Further, if a DVA was seen, a potential microbleed in the vicinity was not rated as it could represent a small cavernoma. WMH were rated on axial fluid attenuated inversion recovery (FLAIR) sequences, and defined as 0 = none or single punctate, 1 = multiple punctate, 2 = early confluent, 3 = large confluent, according to the Fazekas scale[42]. Lacunes were defined as 3-15mm in size, with CSF signal on T2, FLAIR and T1.

In study I, images were analyzed by Sara Shams, a trained rater and at that time an MD/PhD student, for CMBs. All images were analyzed in a blinded manner without knowledge of patient data; additionally intra-rater agreement was performed. Inter-rater agreement was done blinded by Juha Martola, a neuroradiology attending, on 100 randomly selected patients in the cohort.

In study II, three raters were chosen, Sara Shams, by then an MD/PhD student, Juha Martola and Lena Cavallin, both attending neuroradiologists. All raters did a blinded, randomized and independent analysis of CMBs according to the MARS on a T2*, SWI and a reformatted thick slice SWI. For all raters, first CMBs on the T2* were rated, three days later CMBs on the SWI were rated and six months later on the tSWI. Images were randomized between each

rating, and ratings were done in a blinded manner and continuously over a single day. Additionally WMH analysis was performed according to the Fazekas scale, as defined above[42].

In study III and IV, Sara Shams and Juha Martola jointly (i.e. by consensus) analyzed all images for CMBs, lacunes and WMH.

3.5 CEREBROSPINAL FLUID ANALYSIS

Lumbar puncture was done as part of the memory clinic investigation. CSF was collected in 10 ml polypropylene tubes, and centrifuged within 2 h, at 1900g for 10 min, and then frozen until analysis. Biomarkers measured were Aβ 42 (Innotest b-amyloid (1–42)), T-tau (Innotest hTau-Ag) and P-tau (Innotest Phospho-tau (181 P) (Innogenetics, Ghent, Belgium), all measured with sandwich type enzyme-linked immunosorbent assay. Blood samples were collected at the same time as lumbar puncture for analysis of the CSF/serum albumin ratio. All analyses were done at the department of clinical chemistry, Karolinska university hospital, Stockholm, Sweden. The team involved in the analyses was unaware of the diagnoses and study hypotheses.

3.6 STATISTICAL ANALYSIS

General: Descriptive variables are presented as means (±SD) for parametric variables and median and interquartile range (IQR) for nonparametric data. $P<0.05$ was set as the threshold of statistical significance. SPSS 22.0 was used for statistical analysis.

Study I: Chi-squares and Fisher's exact test were used in the assessment of categorical data. Mann-Whitney U and the Kruskall-Wallis tests were used for continuous nonparametric data. Logistic regression analysis was used with CMBs dichotomized into present/absent as a dependent variable and diagnosis, with SCI as a reference, as an independent variable. The prior model was adjusted for hypertension, hyperlipidemia, diabetes, sex, age, MRI field strength (1.5/3.0T) and CMB sequence (SWI/T2*). Another logistic regression model was created to assess the impact of risk factors on CMBs. CMBs (present/absent) were used as a dependent variable, with the number of risk factors used as an independent variable. A third negative binomial regression model was constructed, and CMBs numbers/ numbers in different topographies were used as a dependent variable and risk factors as independent variables. The both two latter models were adjusted for MRI field strength (1.5/3.0T) and CMB sequence (SWI/T2*). Bonferroni correction was applied for each P-value.

Study II: McNemar and Wilcoxon signed rank tests were used to determine the differences between prevalence and number of CMBs, between sequences. Intra class correlation coefficient analysis was made for inter-rater agreement. Chi-squares were used for categorical data and Mann-Whitney U tests for continuous data. Negative binomial regression models were constructed with numbers of CMBs, for the different sequences, as dependent variables, and clinical parameters as independent variables. The models were subsequently corrected for age and sex. Bonferroni correction was applied for each P-value.

Study III: CSF-biomarkers were log-transformed to reach normal distribution. Analysis of variance (ANOVA) with post-hoc Bonferroni correction was used for comparison of CSF biomarkers in patients with zero, one and multiple (arbitrarily defined as six or more) CMBs. Multivariate linear regression analysis was used to determine associations between CSF biomarkers (dependent variable) and CMBs, WMH and lacunes as independent variables; the model was adjusted for MRI field strength (1.5/3.0T), CMB sequences (SWI/T2*), age and sex.

Study IV: CSF-biomarkers were similarly log-transformed to reach normal distribution. Multivariate linear regression models with CSF biomarkers as dependent variables and CMBs, dichotomized into present/absent for different topographies, as independent variables. Multivariable logistic regression models with CMBs in different locations (deep/lobar) and probable CAA-related/unrelated were used as dependent variables and WMH, lacunes, CSF Aβ 42, T-tau, P-tau were used as independent variables. The model was adjusted for age, hypertension, diagnosis, MRI field strength (1.5/3.0T) and CMB sequence (SWI/T2*). Bonferroni correction was applied for each P-value.

4 RESULTS

4.1 STUDY I

Prevalence: CMBs had a 22% prevalence (332/1504) in our memory clinic cohort. They were most common in patients with VaD, 59%, followed by 40% in ARD, 33% in unspecified dementia, 28% in AD, 24% in Pakinson's dementia, 21% in MCI, 19% in other disorders, 17% in FTD, 13% in asymptomatic hereditary dementia and 11% in SCI. The prevalence and number ($P<0.001$) as well as presence of multiple CMBs ($P=0.03$) varied significantly between the different diagnoses. The odds of having CMBs in the different diagnostic groups are seen in Table 3.

Topography: The most common location for CMBs in the whole cohort was lobar (84%), followed by infratentorial (30%), and deep (27%). In the separate diagnoses only AD had CMBs more significantly in the brain lobes ($P=0.01$), with the occipital lobe being the most common location in detailed analysis ($P=0.007$).

Clinical associations: CMBs were significantly more frequent in male patients ($P<0.001$), patients with high age ($P<0.001$), and hypertension ($P<0.001$). MMSE scores were lower in patients with CMBs than without ($P=0.02$). In the separate diagnostic groups CMB prevalence was higher with hypertension in SCI ($P=0.02$), hyperlipidemia in MCI ($P=0.03$), male sex in AD, MCI and VaD ($P<0.05$), and high age in AD and MCI ($P<0.05$). In binomial negative regression analysis, with number of CMBs as a dependent variable, it was seen that male sex (in whole cohort, AD, MCI, VaD), high age (in whole cohort, AD, MCI) and hypertension (in whole cohort, SCI) were associated with increased numbers of CMBs ($P<0.05$). Hyperlipidemia (in whole cohort, MCI, VaD) and diabetes (in all large groups) were associated with a lower number of CMBs ($P<0.001$). Varying topography of CMBs was associated with different risk factors: hypertension was associated with high number of

CMBs in both lobar (in whole cohort, SCI) and deep/infratentorial locations (in whole cohort, AD, MCI) (P<0.05). Hyperlipidemia was associated with lower number of CMBs in deep/infratentorial locations in the whole cohort (P<0.05). Low CMBs in both lobar and deep/infratentorial locations were associated with diabetes in MCI (P<0.05). Male sex was associated with high number of CMBs in lobar (AD) and deep/infratentorial regions in the whole cohort, and MCI, and with deep/infratentorial only in VaD (P<0.05). Infratentorial/deep and lobar (whole cohort and MCI; only lobar: AD) CMBs were associated with high age (P<0.05).

Table 3. Odds ratios for CMBs in the different diagnoses.

Diagnosis	OR for CMBs (95%CI)	Adjusted OR for CMBs (95%CI)
Subjective cognitive impairment (n=385)	1.0 (Ref.)	1.0 (Ref.)
Alcohol related dementia (n=20)	5.5 (2.1-14.2)[a]	4.0 (1.4-11.2)[b]
Alzheimer's disease (n=423)	3.2 (2.2-4.7)[a]	2.0 (1.2-3.1)[b]
Asymptomatic hereditary dementia (n=45)	1.3 (0.5-3.2)	1.5 (0.5-4.1)
Frontotemporal lobe dementia (n=30)	1.6 (0.6-4.5)	1.2 (0.4-3.4)
Mild cognitive impairment (n=418)	2.2 (1.5-3.3)[a]	1.5 (0.9-2.3)
Other disorders (n=53)	2.1 (0.9-4.4)	1.2 (0.5-2.8)
Parkinson's Dementia (n=21)	2.6 (0.9-7.4)	1.7 (0.5-5.7)
Unspecified dementia (n=55)	3.1 (1.7-6.0)[a]	2.2 (1.0-4.4)[a]
Vascular dementia (n=54)	10.9 (6.0-19.7)[a]	10.9 (6.0-19.7)[a]

Adjustment is made for hypertension, hyperlipidemia, diabetes, sex, age, MRI field strength and CMB sequence.[a]P<0.001, [b]P<0.01

4.2 STUDY II

Prevalence/number of CMBs across sequences: On T2* the prevalence was 17% (43/246), on SWI 21% (51/246) and 20% (50/246) on thick slice SWI. The difference in prevalence and number of CMBs was significant when comparing SWI and T2* (P<0.05), and for number of CMBs between thick slice SWI and T2*. There was no significant difference between conventional thin slice SWI and thick slice SWI. Results were correspondingly similar in the different diagnoses.

Inter-rater agreement: Inter-rater agreement across three raters with varying experience was excellent across sequences. Reasons for disagreement that were identified were CMBs close to vessels, lack of attention by the rater, multiple, pale and small CMBs.

Clinical relevance: In univariate analysis of patients with and without CMBs an association was seen between patients with CMBs and high age (P=0.01) and high WMH score (P=0.006). The controlled negative binomial regression analyses showed an association between increased number of CMBs and AD, MCI, other dementias, age, male sex, current alcohol drinking, heredity for dementia, and increased WMH burden for all sequences

(P<0.05). It was further seen that low number of CMBs was associated with anticoagulants, current smoking and hyperlipidemia. Only minor differences across sequences were noted.

4.3 STUDY III

CMBs and associations with CSF biomarkers: Patients with 0, 1 and ≥6 CMBs were compared. Aβ42 levels were significantly lower in patients with ≥6 CMBs when compared to patients with 1 or 0 CMBs (P<0.01). Aβ42 levels were significantly lower when comparing ≥6 and 0 CMBs, in AD and MCI (P<0.05). In AD, as well as in AD and VaD with hypertension, T-tau and P-tau levels were lower in patients with ≥6 CMBs when compared to 0 (P<0.05). CSF/serum albumin ratios were higher in patients with ≥6 CMBs when compared to 0 (P<0.001), in the whole cohort.

Independent associations of CMBs with CSF biomarkers: In multivariate linear regression analysis with CMBs, WMH and lacunes in the same model, it was seen that CMBs were associated with low Aβ42 levels (P<0.01) in the whole cohort, AD and MCI. WMH were associated with low Aβ42 levels in the whole cohort and in AD (P<0.05). T-tau was high with increased WMH in the whole cohort and low in AD (P<0.05). CSF/serum albumin ratios were high with increased WMH. Aβ42 levels were high with lacunes in the whole cohort (P≤ 0.05).

4.4 STUDY IV

Topographies and associations with CSF biomarkers: Lobar CMBs were associated with low Aβ42 levels in the whole cohort, AD, MCI (P<0.05). P-tau showed a tendency to be lower with infratentorial CMBs in AD. No other associations were found. In more detailed brain topographies Aβ42 levels showed a tendency to be high with CMBs in the cerebellum in AD and the whole cohort (P<0.05, before Bonferroni correction). CMBs in the brainstem showed associations with low T-tau, P-tau, in the whole cohort and AD (P<0.05, before Bonferroni correction), and with high CSF/serum albumin ratio in MCI (P<0.05). Deep CMBs were associated with lower T-tau levels (P<0.05, before Bonferroni correction). Frontal CMBs were associated with low Aβ42 levels AD (P<0.001), and showed a tendency to be low in SCI (P<0.05, before Bonferroni correction). Temporal CMBs showed a tendency to be associated with low Aβ42 levels in the whole cohort and MCI (P<0.05, before Bonferroni correction). Occipital CMBs showed an association with low Aβ42 levels in the occipital lobe (P<0.01), AD and MCI (P<0.05, before Bonferroni correction), and higher CSF/serum albumin ratios in AD (P<0.05, before Bonferroni correction).

Prediction of CMBs through imaging and CSF biomarkers: Data is displayed in Table 4, below.

Table 4. Prediction of different types of CMBs by CSF and imaging biomarkers

(OR; 95%CI; p-value)	Lobar CMBs	Deep CMBs	Probable CAA-related CMBs (vs. no CMBs)	Probable CAA unrelated CMBs (vs. no CMBs)
WMH	1.68 (1.35-2.09); P<0.0001	2.97 (2.08-4.25); P<0.0001	1.86 (1.27-2.73); P=0.002	1.78 (1.44-2.20); P<0.0001
Lacunes	2.61 (1.69-4.04); P<0.0001	1.93 (0.98-3.80); P=0.059	4.11 (1.86-9.10); P<0.0001	1.92 (1.22-3.04); P=0.005
CSF Aβ42	0.11 (0.04-0.29); P<0.0001	0.38 (0.07-2.14); P=0.274	0.01 (0.00-0.03); P<0.0001	0.51 (0.19-1.35); P=0.172
CSF T-Tau	0.61 (0.23-163); P=0.325	0.25 (0.05-1.23); P=0.089	0.94 (0.11-8.36); P=0.959	0.70 (0.26-1.89); P=0.484
CSF P-tau	2.06 (0.61-6.91); P=0.244	2.72 (0.39-19.05); P=0.311	0.83 (0.06-11.08); P=0.888	2.07 (0.60-7.12); P=0.247

5 DISCUSSION

Study I: We show that CMBs are common in a memory clinic and that risk factor associations differ depending on diagnosis and topography. Our prevalence is in line with what other studies have shown[15], as seen in Table 5.

Table 5. Prevalence of CMBs across different cohorts of cognitive impairment.

Study	Prevalence, % (n)	MRI Field Strength (T)	Sequence
[68]	33 (6)	3.0	T2*
[69]	45 (66)	3.0	T2*
[70]	26 (98)	3.0	T2*
[71]	39 (26)	3.0	T2*
[72]	12 (25)	3.0	T2*
[73]	20 (32)	3.0	T2*
[74]	33 (79)	3.0	SWI
[75]	43 (17)	3.0	SWI
[76]	48 (77)	3.0	SWI
[73]	22 (35)	3.0	SWI
[77]	28 (118)	3.0-1.5	SWI &T2*
[78]	21 (117)	3.0–1.0	T2*
[79]	20 (16)	1.5	T2*
[50]	20 (10)	1.5	T2*
[55]	32 (19)	1.5	T2*
[56]	16 (8)	1.5	T2*
[57]	18 (7)	1.5	T2*
[80]	24 (132)	1.5	T2*
[81]	18 (21)	1.5	T2*
[58]	18 (23)	1.5	T2*
[82]	33 (24)	1.5	T2*
[50]	39 (19)	1.5	SWI

CMBs were most common in VaD in our cohort, which is rational, due to the amount of vascular risk factors in VaD. This was followed by a high prevalence of CMBs in ARD, which also makes sense, due to the alcohol related vascular risk factors such as diabetes, hypertension and hyperlipidemia. AD had a 28% prevalence, one of the highest in our cohort, which may be explained by the large amount of CAA in AD, almost all patients with AD thought to have CAA[25,26].

Lobar CMBs being most common in AD is also reasonable, due to the high amount of CAA in AD. Further, occipital CMBs being most common may be explained by the fact that CAA favors the brain lobes, and seems to have a predilection for the occipital lobe. Variance of risk factors with CMBs is further expected, due to the fact that deep and infratentorial CMBs are thought to represent hypertensive arteriopathy, and lobar CMBs CAA. Hypertension was however associated with both lobar and deep CMBs, which may suggest an overlap between CAA and hypertensive arteriopathy in our cohort. Male sex also showed an association with both infratentorial/deep and lobar CMBs, suggesting that CMBs, perhaps due to the increased vascular risk factors, are more common in male, as has been shown in another study[83].

General risk factor associations with CMB presence, independent of location, were hypertension, high age and male sex. MMSE was lower in patients with CMBs, in the whole cohort, in univariate analysis, most probably due to the high amount of CMBs in VaD and AD. CMBs and the possible impact on cognition is however not well investigated, and studies have shown varying results[58,84–87]. Increasing number of risk factors lead to higher OR for CMBs, showing that the risk of CMBs is at highest with accumulated risk factors.

The limitations in our study include three different scanners and both T2* and SWI sequences used, although our regression models were corrected for these two variables. Advantages include a large cohort, representative of a typical memory clinic panorama, with thorough imaging analysis and memory clinic investigation.

Study II: Our study shows that SWI increases the number and prevalence of detected CMBs. SWI is consequently, due to this fact, the recommended sequence to use in CMB detection. We note however that CMB detection is excellent across raters of different experience and sequences, and that clinical associations remain the same. Consequently, studies on CMBs are comparable with regard to clinical associations.

Prior studies have shown favorable results promoting the SWI sequence[48,50,88], although no difference has been noted in clinical associations between T2* and SWI[50]. Inter-rater agreement has been excellent throughout sequences in one study[50], as well as better on SWI[49]. Prevalence and number of CMBs both increase with the use of SWI[49,50]. We, and Goos et al used a thicker reformatted SWI in order to differentiate the effect of the routinely thinner slice thickness in SWI vs. the intrinsic contrast enhancing properties of SWI. Thick SWI showed an increase in number and prevalence of CMBs detected, and the difference between thick and thin SWI was minor. Thin SWI, however, showed the highest increase in CMBs, which most certainly is due to the thinner slice thickness effect of SWI.

Clinical associations were shown between CMBs and AD, MCI, and other dementias, which are all rational as seen in Study I. More surprisingly we saw the association of hyperlipidemia and lower number of CMBs, which is similar to that seen in Study I, this may represent a population well treated in terms of vascular risk factors, which in turn would explain the association with lower number of CMBs. Further surprising was the association between smoking and lower number of CMBs, similar to what has been shown prior[50,89].
Higher numbers of CMBs were associated with alcohol consumption, which is rational considering the high prevalence of CMBs in ARD.

Strengths of our study include a large cohort, three raters with varying experience, and thorough rating methods with the standardized MARS. Phase or quantitative susceptibility maps may have further aided in the correct differentiation of CMBs and calcifications.

Study III: We show that increased number of CMBs is associated with decreased levels of Aβ42, indicating increased amyloid deposition in the brain[36]. CSF/serum albumin ratios were increased with higher number of CMBs, most probably indicating a disrupted BBB. T-tau and P-tau were lower with higher number of CMBs, suggesting a lack of association between neurodegeneration and NFTs with CMBs.

Prior studies have similarly shown an association between CMBs and amyloid deposition in the brain, reflected by low CSF Aβ42 or PET amyloid imaging[65,78,90–92]. The association with T-tau and P-tau is less clear. Patients with multiple CMBs (≥8) have shown higher P-tau and T-tau levels when compared to patients with zero CMBs[91]. This is contrary to our results, however differentiating based on CMB location might yield different data. Similarly, in study IV we showed an association between increased deep/infratentorial CMBs and low T-tau and P-tau, whereas Chiang et al showed high P-tau levels with lobar CMBs[85]. This makes sense as we show low T-tau and P-tau levels with deep/infratentorial CMBs in AD as well as in AD and VaD with hypertension. In patients with hypertension there was no association between Aβ42 and CMBs, further supporting the fact that hypertensive arteriopathy is different from amyloid pathology and the associated neurodegeneration.

WMH was also related to lower CSF Aβ42 levels in independent regression analysis. WMH has shown to predict AD and cognitive impairment independently[93–95], and the association seen is rational. High CSF/serum albumin ratio with WMH may indicate that WMH is associated with a disruption of the BBB[30]. High Aβ42 levels were associated with lacunes, suggesting that lacunes mostly are associated with hypertensive arteriopathy, and are not as often related to CAA[30].

Strengths of our study include a large cohort, thorough diagnosis and imaging ratings. Limitations include the use of different MRI scanners and field strengths, which however were controlled for in our regression analysis. Further, as diagnoses was set in multidisciplinary rounds with access to all data this may have led to circular reasoning and classification of patients in diagnostic groups based on for instance imaging findings.

Study IV: We show that lobar CMBs are associated with low Aβ42 levels and that deep/infratentorial are associated with high Aβ42 levels. This corroborates the theory of region based SVD, with lobar CMBs representing CAA and deep/infratentorial CMBs hypertensive arteriopathy.

There are to date few studies on the topography of CMBs and associations with CSF biomarkers. Lobar CMBs are associated with higher amyloid in the brain, reflected either by CSF or PET imaging[85,96,97]. P-tau has shown to be higher with CMBs in lobar brain regions[85], and it is rational that CAA and the accompanying neurodegeneration would be associated with elevated tau levels. In more detailed topographies, especially occipital CMBs showed an association with lower Aβ42 levels and a high CSF/serum albumin ratio; this is reasonable since CAA has a predilection for the occipital lobe[27,65,98]. Meanwhile deep/infratentorial CMBs show associations with high Aβ42 and low T-tau and P-tau levels, and this supports the idea of differing underlying pathology of hypertensive arteriopathy. Aβ42 levels predicting lobar CMBs as well as CAA-related and -unrelated CMBs, but not deep, additionally support the association of amyloid and lobar CMBs. Suggestively, lobar CMBs are of more importance to study, considering the relation to CAA/neurodegeneration in cognitive impairment, although CMBs due to hypertensive arteriopathy may have an overlapping additional effect.

Strengths of our study include a large cohort, thorough diagnosis and imaging analysis. Negatives are the use of different MRI field strengths as well as CMB sequences, which however were corrected for in the different analyses.

General: Cerebral microbleeds are a common marker in cognitive impairment. The associations shown in cognitive impairment suggest that mainly lobar CMBs, i.e. CAA, is of importance in cognitive impairment, although hypertensive arteriopathy may have a synergistic contributing effect. It is however important to consider all markers of SVD, as detailed in figure 8, and the independent role of CMBs in this spectrum. The pattern of associations and their timely manifestation in cognitive impairment is another important aspect, necessitating further investigation. If SVD is a significant part of cognitive impairment, it should, similarly to disease pathophysiology, be evident in the preclinical

stages of disease. It is also important to realize the overlap between cognitive impairment and small vessel disease, and the fact that it may be hard to tease apart the respective effects.

CAA being most important, and especially involved in AD pathology, is reflected by our data with association to CSF biomarkers, and the lack of association when including hypertensive arteriopathy. However hypertensive arteriopathy may still cause neurodegeneration and brain related damage on its own.

6 CONCLUSIONS

Study I: CMBs are common in a memory clinic and show varying risk factor associations and topography depending on underlying dementia diagnosis.

Study II: SWI has higher sensitivity for CMBs compared with T2*, and on the basis of this we recommend SWI as the sequence for CMB detection. Inter-rater agreement is excellent across sequences. Clinical associations with CMBs are comparable across SWI and T2*, suggesting that studies on CMBs using differing sequences can be compared.

Study III: CMBs are primarily associated with lower CSF Aβ42 levels, and there is an accumulating effect with increased number of CMBs. CSF/serum albumin ratios are high with CMBs, possibly reflecting a disrupted blood brain barrier. There is no effect on T-tau and P-tau.

Study IV: Lobar CMBs are associated with low CSF Aβ42 levels, and deep/infratentorial with lower T-tau and P-tau levels. Our study supports the theory of lobar CMBs being associated with CAA and deep/infratentorial with hypertensive arteriopathy.

7 FUTURE ASPECTS

SVD, and the accompanying imaging markers, have long been overlooked and considered a normal finding in the brain. The implications, associations and longitudinal outcomes of SVD are however in need of more investigation, not only in cognitive impairment, but also in patients with stroke and cardiovascular disease.

This thesis has been the start of the KIDS, a series of studies on dementia and imaging markers, to help aid and better understand dementia and its progression from cognitive impairment. We have to date multiple studies on the same topic that are accepted, in manuscript, or currently being pursued. Our future studies aim to determine the effects of all SVD imaging markers on cognitive impairment, as well as in patients with stroke. We further aim to longitudinally study SVD and the outcomes in both these groups. SVD is still a field where there is uncertainty regarding the effects and implications, as well as current treatment, and we aim to fill the knowledge gap by our research.

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